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Diversity of Tetracycline Resistance Genes in Bacteria from Chilean Salmon Farms

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Twenty-five distinct tetracycline-resistant gram-negative bacteria recovered from four Chilean fish farms with no history of recent antibiotic use were examined for the presence of tetracycline resistance (*tet*) genes. Sixty percent of the isolates carried 1 of the 22 known *tet* genes examined. The distribution was as follows. The *tet*(A) gene was found in six isolates. The *tet*(B) gene was found in two isolates, including the first description in the genus *Brevundimonas*. Two isolates carried the *tet*(34) and *tet*(B) genes, including the first description of the *tet*(34) gene in *Pseudomonas* and *Serratia* and the first description of the *tet*(B) gene in *Pseudomonas*. The *tet*(H) gene was found in two isolates, which includes the first description in the genera *Moraxella* and *Acinetobacter*. One isolate carried *tet*(E), and one isolate carried *tet*(35), the first description of the gene in the genus *Stenotrophomonas*. Finally, one isolate carried *tet*(L), found for the first time in the genus *Morganella*. By DNA sequence analysis, the two *tet*(H) genes were indistinguishable from the previously sequenced *tet*(H) gene from Tn*5706* found in *Pasteurella multocida*. The *Acinetobacter radioresistens* isolate also harbored the Tn*5706*-associated 1,063-bp IS element IS*1597*, while the *Moraxella* isolate carried a 1,026-bp IS-like element whose 293-amino-acid transposase protein exhibited 69% identity and 84% similarity to the transposase protein of IS*1597*, suggesting the presence of a novel IS element. The distribution of *tet* genes from the Chilean freshwater ponds was different than those that have previously been described from other geographical locations, with 40% of the isolates carrying unidentified tetracycline resistance genes.

Intensive fish farming is done in Chile, which is the second-largest producer of farm-raised salmon in the world (25). Oxytetracycline is the most frequently used antimicrobial agent in the Chilean salmon industry, which has resulted in increased tetracycline resistance (Tc^r) in gram-negative bacteria associated with all aspects of fish farming, from the water entering and leaving the ponds to the fish food pellets themselves. Previous studies of Tc^r *Acinetobacter* spp., *Aeromonas hydrophila*, *Edwardsiella tarda*, *Pasteurella piscicida* (which has been reclassified as *Photobacterium damselae* subsp. *piscicida*) (7), *Vibrio anguillarum*, and *Vibrio salmonicida* from fish farms have been characterized in other geographical areas (2, 8, 23). A few studies have characterized nonpathogenic bacteria isolated from catfish ponds (5) or from polluted and unpolluted marine sediments (1). In these previous studies, 66 to 94% of the total isolates carried one of five known Tc^r genes: *tet*(A), *tet*(B), *tet*(C), *tet*(D), and *tet*(E).

A recent report describes the isolation of Tc^r bacteria from fish farm influents, salmon culture tanks, farm effluents, surface water, salmon, and unmedicated fish food pellets (15). From that study, 25 Tc^r isolates were selected for characterization of the tetracycline resistance genes by using oligonucleotide probes representing 23 of the known tetracycline resistance genes (3). In selected cases, mating and transformation experiments were done, and *tet* genes were sequenced.

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MATERIALS AND METHODS

Bacteria. Originally, 103 oxytetracycline-resistant gram-negative isolates were recovered from four freshwater Chilean salmon farms located in the southern part of the country (14). From this collection, 25 isolates were obtained that represented all four fish farms and the various locations of sample collection (fish farm influents, salmon culture tanks, farm effluents, surface water, salmon fingerlings, and unmedicated fish food pellets). In particular, isolates representing genera not previously examined for *tet* genes were also included. These isolates had previously been identified and included *Acinetobacter* spp. (4), *Aeromonas hydrophila* (1), *Brevundimonas vesicularis* (2), *Escherichia coli* (1), *Enterobacter sakazakii* (1), *Moraxella* sp. (1), *Morganella morganii* (1), *Pseudomonas fluorescens* (4), *Pseudomonas* sp. (3), *Pantoea* sp. (1), *Providencia rettgeri* (1), *Ralstonia pickettii* (1), *Serratia liquefaciens* (1), *Sphingomonas paucimobilis* (1), and *Stenotrophomonas maltophilia* (1) (Table 2). The tetracycline MICs ranged from 128 to 2,048 μg/ml (14). The isolates were maintained on L agar supplemented with 25 μg of tetracycline per ml at either room temperature (25°C) or at 37°C, depending on the species.

DNA-DNA hybridization. Whole bacteria and whole DNA dot blots were prepared as previously described (13). Twenty-three *tet* gene probes were used for hybridization of whole bacteria dots and whole DNA dots. The specific oligonucleotide probes for *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(G), *tet*(H), *tet*(M/O/S), *tet*(P), and *tet*(Q) have been previously characterized (18–22, 26) (Table 1). We also included some *tet* genes commonly found in gram-positive bacteria as well as some of the newer *tet* genes (Table 1).

MICs. The oxytetracycline MICs were previously done (15). The doxycycline and minocycline MICs were determined by agar dilution following National Committee for Clinical Laboratory Standards (NCCLS) protocols (16), with final concentrations ranging from 2 to 256 μg/ml. Plates were incubated at room temperature for 48 h. *E. coli* reference strain ATCC 25922 was used as a control, incubated at 37°C, and read after 24 h. NCCLS breakpoints for all tetracyclines are defined as follows: susceptible (S), ≤4 μg/ml; intermediate (I), 8 μg/ml; and resistant (R), ≥16 μg/ml (16).

PCR assay. Those isolates positive by DNA-DNA hybridization were verified by PCR assay with hybridization of the PCR products with an internal probe by using previously described PCR assays for the *tet*(A), *tet*(B), *tet*(E), *tet*(L), and *tet*(H) genes (6, 18) and/or by PCR sequencing (Table 1).

TABLE 1. Primers used in this study

Gene	Primer name	Sequence (5′ → 3′)
tet(A)	A1	CGA GCC ATT CGC GAG AGC
	A2	CGA ABC AAG CAG GAC CAT G
	A3	GCC TCC TGC GCG ATC TGG
tet(B)	BF	CAG TGC TGT TGT TGT CAT TAA
	BR	GCT TGG AAT ACT GAG TGT AA
tet(C)	CI	CTT GAG AGC CTT CAA CCC AG
	CR	TGG TCG TCA TCT ACC TGC C
tet(D)	DF	GGA TAT CTC ACC GCA TCT GC
	DR	CAT CCA TCC GGA AGT GAT AGC
tet(E)	EF	TCC ATA CGC GAG ATG ATC TCC
	ER	CGA TTA CAG CTG TCA GGT GGG
tet(G)	G	AAC AAT GTC AGC AGT AAC AAG
tet(H)	HF	ATA CTG CTG ATC ACC G
	HR	TCC CAA TAA GCG ACG C
tet(J)	JF	ATT TAT CTC TGT TTT TGG CAC
	JR	GGC CCG TTA CTC TTC TCT TTT
tet(K)	K	TAA AGT AAT GGT ACC TGG TAA ATC AAC
tet(L)	L	CTT ATC GTT AGC GTG CTG TCA TTC C
	LR	TTA AGC AAA CTC ATT CCA GC
tet(M/O/S)	M6	GTT TAT CAC GGA AGY GCWA
	M4	GAA GCC CAG AAA GGA TTY GGT
tet(P)	P1	CAC AGA TTG TAT GGG GAT TAG G
	P2	CAT TTA TAG AAA GCA CAG TAG C
tet(Q)	MR6	CTG TCC CTA ACG GTA AGG
	MR7	TTA TAC TTC TTC CTC CGG CAT CGG T
tet(T)	TF	GCG TTA AGG GAT CAA ATC TTC C
	TRV	CAG GTA TGC TCC CTC ATC AAC
tet(Y)	YF	CAC TTA TAA CCG CAC TGA TTG
	YR	ATC GCA ATA AAC TGG CAA TGT
tet(Z)	ZF	GCT CCT TCC ATT CTT TCT AAC
	ZR	TGT AGT CAG GCA GGT CAA TGT
tet(30)	30F	CCG GAA ACT GAT TGC ACG TCC GCG
	30R	GCC TTC GGG GTT TTC ACC GTT GCG
tet(31)	31	GAA GGT TTA TAG GTT TAT
tet(34)	34F	ATG AAA ACG AAC GCT AAT TAA CCA
	34R	ACA TAG AGA TCG ATG CTA GTA CTA
	34I	CTA CCG AAT CGC GTT TGT CG
tet(35)	35F	ATG CGC AAG ACC GTC CTAC
	35R	CAC ACA CTA GTA ACG GTC GAA
	35	ATC GAC GCA GCT ATG CAC TA
IS elements	IS	ACC TGA GTT CGG GAT AAG

Transfer experiments. The isolates selected for use as donors included *A. hydrophila* carrying *tet*(E); *Brevundimonas vesicularis* carrying *tet*(B); *Enterobacter sakazakii* carrying *tet*(B); *Pseudomonas fluorescens* carrying *tet*(A); *P. pseudoalcaligenes* carrying *tet*(B); *Serratia liquefaciens* carrying *tet*(B) and *tet*(34); *Moraxella* spp. and *Acinetobacter radioresistens*, both carrying *tet*(H); *Morganella morganii* carrying *tet*(L); *Stenotrophomonas maltophilia* carrying *tet*(35); and *Acinetobacter* sp., *Brevundimonas vesicularis*, *Providencia rettgeri*, and *Ralstonia pickettii* carrying unknown genes (Table 2). Transfer of the tetracycline resistance phenotype was done with the recipient *E. coli* strains HB101 and DH5α, which had been selected for resistance to streptomycin (1,000 μg/ml), nalidixic acid, fusidic acid, and rifampin at 25 μg/ml each and with the Chilean strains as donors as previously described. All matings were done at 37°C a minimum of two times as previously described (5). Selected transconjugants' tetracycline genes were verified by DNA-DNA hybridization and/or PCR. Experiments involving transformation into *E. coli* JM107 as well as electrotransformation into tetracycline-susceptible *Mannheimia haemolytica* M3000 and *Pasteurella multocida* P4000 strains were performed as described previously (11). Selection of the transformants was done on Luria-Bertani agar or sheep blood agar supplemented with 20 μg of tetracycline per ml.

Partial sequence of the *tet*(L) PCR product. The *tet*(L) PCR product was sequenced and compared by using the Biological Information's Resource Software at the University of Washington as previously described (13).

Sequencing and location of *tet*(H) genes and Tn*5706*-associated IS elements. The *tet*(H) genes and the Tn*5706*-associated IS elements were amplified by PCR. For *tet*(H) amplification, the previously described primers (Table 1) were used, which resulted in a 1,076-bp internal segment of the *tet*(H) gene. For amplification of the IS elements, a single 18-bp oligonucleotide that corresponded exactly to the perfect 18-bp inverted repeats at the termini of IS*1596*/IS*1597* was used. The PCR products were cloned into pCR-Blunt II-TOPO (Invitrogen, Groningen, The Netherlands). The cloned PCR amplicon of the novel IS*1599* element was used as a specific gene probe in subsequent hybridization experiments. Confirmation of the plasmid location of the *tet*(H) genes and the IS*1597* and IS*1599* elements was achieved by Southern blot hybridization experiments. For this, plasmid profiles of the *Acinetobacter* and *Moraxella* isolates were prepared with the Qiagen midi kit (Qiagen, Hilden, Germany).

Nucleotide sequence accession number. The sequences of the *tet*(H) amplicons and the IS elements of the *Acinetobacter* and *Moraxella* isolates have been deposited in the EMBL database under the following accession numbers: *Acinetobacter tet*(H), AJ487672; *Moraxella tet*(H), AJ487674; *Acinetobacter* IS*1597*, AJ487673; and *Moraxella* IS*1599*, AJ487675.

RESULTS

MICs. Twenty-two (88%) of the 25 isolates were doxycycline resistant (≥16 μg/ml), while 2 of the 3 remaining isolates showed intermediate resistance (8 μg/ml), and 1 isolate was susceptible to doxycycline (Table 2). Of the four isolates carrying the *tet*(B) gene, three were minocycline resistant (16 μg/ml), with *E. sakazakii* being intermediate to minocycline (8 μg/ml). Only one other isolate, the *A. hydrophila* strain carrying a *tet*(E) gene, was resistant to minocycline, while two *P. fluorescens* isolates carrying a *tet*(A) gene and one *R. pickettii* isolate carrying an unknown gene were also intermediate to minocycline (Table 2).

Distribution of the *tet* genes. Initially the 25 isolates were examined for the presence of *tet*(A) through *tet*(G), since these genes have previously been found in bacteria isolated from freshwater ponds (4, 5, 8, 23). Only three of the six *tet* genes were found in the isolates, and 11 (44%) of the 25 isolates were positive for the *tet*(A) gene (4 *Pseudomonas fluorescens* isolates, 1 *Pseudomonas* sp. isolate, and 1 *E. coli* isolate); the *tet*(B) gene (1 *Brevundimonas vesicularis* isolate, 1 *Enterobacter sakazakii* isolate, 1 *Pseudomonas pseudoalcaligenes* isolate, and 1 *Serratia liquefaciens* isolate), and the *tet*(E) gene (1 *Aeromonas hydrophila* isolate). The presence of these *tet* genes in the respective isolates was verified by PCR assays. The isolates carrying the *tet*(A) gene were from three of the four fish farms, while those with the *tet*(B) gene were from two of the fish farms (Table 2).

The isolates were then screened for 17 additional *tet* genes with probes for *tet*(H) to *tet*(Z), except for *tet*(V) and *tet*(U), *tet*(30), *tet*(31), *tet*(34), and *tet*(35) (Table 1). These *tet* genes were chosen because (i) we had cloned controls available in the

TABLE 2. Characterization of Chilean gram-negative bacteria

Farm	Strain	MIC (μg/ml)[a]			Species	Known *tet* gene	Mobility
		Oxytetracycline	Doxycycline	Minocycline			
CC1	O111	>1,024	64	8	*Pseudomonas fluorescens*	A	No
	O135	512	64	16	*Aeromonas hydrophila*	E	No
	O150	1,024	8	4	*Pseudomonas fluorescens*	A	No
	O152	1,024	32	8	*Pseudomonas fluorescens*	A	No
	O193	1,024	32	8	*Ralstonia pickettii*	None	No
	O205	1,024	32	4	*Pseudomonas putida*	None	Yes
	O213	1,024	256	4	*Acinetobacter* sp.	None	No
	O245	1,024	32	4	*Moraxella* sp.	H	No
	O275	1,024	64	4	*Pseudomonas fluorescens*	None	ND[b]
CC2	C8	128	32	4	*Sphingomonas paucimobilis*	None	ND
	C11	1,024	16	4	*Morganella morganii*	L	No
	CH3	2,048	256	32	*Serratia liquefaciens*	B, 34	Yes, both genes
	CH50	512	256	32	*Pseudomonas pseudoalcaligenes*	B, 34	Yes, both genes
	CH90	512	32	4	*Acinetobacter johnsonii*	None	ND
	CH100	512	16	<2	*Stenotrophomonas maltophilia*	35	Yes, but not *tet*(35)
CC3	Q40	2,048	32	4	*Pseudomonas* sp.	A	ND
	Q52	1,024	64	4	*Pantoea* sp.	None	ND
	Q61	512	256	4	*Providencia rettgeri*	None	Yes
	Q73	1,024	16	<2	*Escherichia coli*	A	ND
	Q75	1,024	8	<2	*Acinetobacter lwoffi*	None	ND
CC4	L7	2,048	32	<2	*Brevundimonas vesicularis*	None	No
	L16	256	32	4	*Pseudomonas fluorescens*	A	No
	L21	512	32	8	*Enterobacter sakazakii*	B	Yes, but not *tet*(B)
	L32	128	4	<2	*Acinetobacter radioresistens*	H	No
	L53	1,024	64	16	*Brevundimonas vesicularis*	B	Yes

[a] The NCCLS breakpoints for all tetracyclines are ≤4 μg/ml = S, 8 μg/ml = I, and ≥16 μg/ml = R (16).
[b] ND, not done.

laboratory that served as positive controls, (ii) they represented the majority of *tet* genes currently characterized, or (iii) they have recently been described in other water bacteria (3, 17, 24). Six isolates hybridized with these additional probes. One isolate each of *Moraxella* sp. and *A. radioresistens* carried the *tet*(H) gene, and these isolates were from two different farms. An *S. liquefaciens* isolate and a *Pseudomonas pseudoalcaligenes* isolate, each carrying *tet*(B) and *tet*(34), were from the same farm. We also identified an *M. morganii* isolate carrying the *tet*(L) gene and an *S. maltophilia* isolate carrying the *tet*(35) gene. All six of these isolates carried multiple plasmids. Additional oligonucleotide probes were used to verify the presence of the *tet*(34) and *tet*(35) genes. The presence of the *tet*(L) and *tet*(H) genes was verified by PCR, Southern blotting, and sequence analysis of the PCR products. All three genes appeared to be associated with plasmids, as confirmed by hybridization. The *tet*(L) PCR product showed 100% amino acid homology with the *tet*(L) gene from the plasmid pTHT15 from *Bacillus stearothermophilus* (data not shown). The two isolates with the *tet*(H) genes are described below. The isolates that did not carry one of the known genes were found in similar numbers from all four farms.

Mobility of the *tet* genes. Selected isolates were used as donors in mating experiments. We were unable to transfer the *tet*(H) from either the *A. radioresistens* or *Moraxella* sp. donors using *E. coli* or *Pasteurella* as recipients, although the genes were associated with plasmids. Similarly, transfers of the *tet*(L) gene from *M. morganii*, the *tet*(A) genes from four different *P. fluorescens* isolates, the *tet*(E) gene from *A. hydrophila*, and the unknown genes from Acinetobacter sp., *A. radioresistens*, *R. pickettii*, and *B. vesicularis* were not detected, although the rate of transfer could be $<1 \times 10^{-10}$ per recipient (data not shown). The *P. pseudoalcaligenes* and *S. liquefaciens* isolates carrying both *tet*(B) and *tet*(34) genes transferred both genes to the *E. coli* recipient at frequencies of 5.0×10^{-5} to 1.3×10^{-6} per recipient, respectively. The 10 individual transconjugants examined received both genes from both matings. The *B. vesicularis* isolate carrying the *tet*(B) gene and the *P. rettgeri* isolate with the unknown *tet* gene transferred at frequencies 1.0 $\times 10^{-6}$ to 9.6×10^{-6} per recipient. The *S. maltophilia* isolate with the *tet*(35) gene transferred tetracycline resistance at similar frequencies, and multiple plasmids were transferred. However, the resulting transconjugants did not carry the *tet*(35) gene. Similarly, the *E. sakazakii* isolate carrying the *tet*(B) gene transferred tetracycline resistance, but the transconjugants did not carry the *tet*(B) gene (Table 2).

Hybridization studies revealed the location of the *tet*(H) genes in the *Moraxella* and *A. radioresistens* isolates on plasmids of less than 12 kb. Since these plasmids are too small for conjugation, transformation into $CaCl_2$-competent *E. coli* strain JM107 and electrotransformation into the recipient strains *Mannheimia haemolytica* M3000 and *Pasteurella multocida* P4000 were repeatedly performed. None of these experiments yielded Tc^r transformants.

Characterization of the Tn*5706*-associated *tet*(H) genes and insertion elements. The *tet*(H) gene has previously been found exclusively in isolates of the two genera *Pasteurella* and *Mannheimia* (9–11). This gene has been well characterized and has

previously been shown to be part of a nonconjugative transposon, Tn*5706*, in which the area from *tetR*(H) to *tet*(H) is bracketed by the almost identical insertion sequences IS*1596* and IS*1597* (12). The *tet*(H)-specific PCR amplicons of the *Acinetobacter* sp. and *Moraxella* sp. isolates were both 1,076 bp in size and proved to be indistinguishable by DNA sequence from the corresponding structural *tet*(H) gene from Tn*5706*. In addition, the *Acinetobacter* isolate also carried the Tn*5706*-associated 1,063-bp insertion sequence IS*1597*. This was confirmed by DNA hybridization and complete sequence analysis of the element. The *tet*(H) gene and IS*1597* element were confirmed by Southern blot hybridization to be located on the same plasmid (data not shown). The *Moraxella* sp. isolate had a slightly smaller amplicon, and sequence analysis identified a 1,026-bp element that closely resembled an insertion sequence. This element, tentatively designated IS*1599*, had the same perfect 18-bp inverted repeats as IS*1596* and IS*1597* at its ends. While IS*1596* and IS*1597* exhibited two open reading frames for proteins of 70 and 228 amino acids (aa), IS*1599* only had a single open reading frame of a 293-aa protein. This protein had 69% identity and 84% similarity to the 228-aa transposase proteins of the aforementioned IS elements. The difference in size between IS*1599* and IS*1596*/IS*1597* is due to the absence of a 34-bp direct repeat located at positions 282 to 315 in IS*1596*/IS*1597*, as well as the loss of a single triplet located at positions 685 to 687 in IS*1596*/IS*1597* (Fig. 1). Hybridization experiments confirmed that the IS*1599* element and the *tet*(H) genes were on the same plasmid in the *Moraxella* sp. isolate.

DISCUSSION

Tetracyclines are the most frequently used antimicrobial agents in veterinary medicine in many parts of the world. Oxytetracycline is the most commonly used antimicrobial in freshwater salmon farming in Chile (14, 15, 24). The spread of *tet* genes is often facilitated by their location on mobile genetic elements, such as plasmids and transposons (5). Of the various *tet* genes currently known, *tet*(A), *tet*(B), *tet*(D), *tet*(E), and *tet*(G) have previously been found in bacteria from fish farms (2, 4, 5, 8, 23). Three of these genes, *tet*(A), *tet*(B), and *tet*(E), were found in 11 (44%) of the isolates from this study. A number of *tet* genes were found in new genera in this study, including the *tet*(L) gene in *Morganella morganii*. The *tet*(L) gene was originally found in various *Bacillus* spp., but more recently it has been described in five other gram-positive genera, *Mycobacterium* spp., and *Streptomyces* spp. and in the gram-negative anaerobes *Fusobacterium* spp. and *Veillonella* spp. (3). However this is the first description of the *tet*(L) gene in a facultative anaerobic species. The detection of *tet*(B) in the genus *Brevundimonas* is also a novel observation (3). This strain was minocycline resistant, as were two of the three other isolates carrying the *tet*(B) gene, which has previously been associated with minocycline resistance (3). The detection of the *tet*(E) gene in *Aeromonas hydrophila* has previously been reported from fish (5). One other isolate, *A. hydrophila*, was resistant to minocycline. Unfortunately, whether this *tet(*E) gene confers minocycline resistance cannot easily be tested, since we could not transfer the gene (Table 2). This was not unexpected, since previously, no one had been able to transfer *tet*(E) genes from other resistant strains and species examined

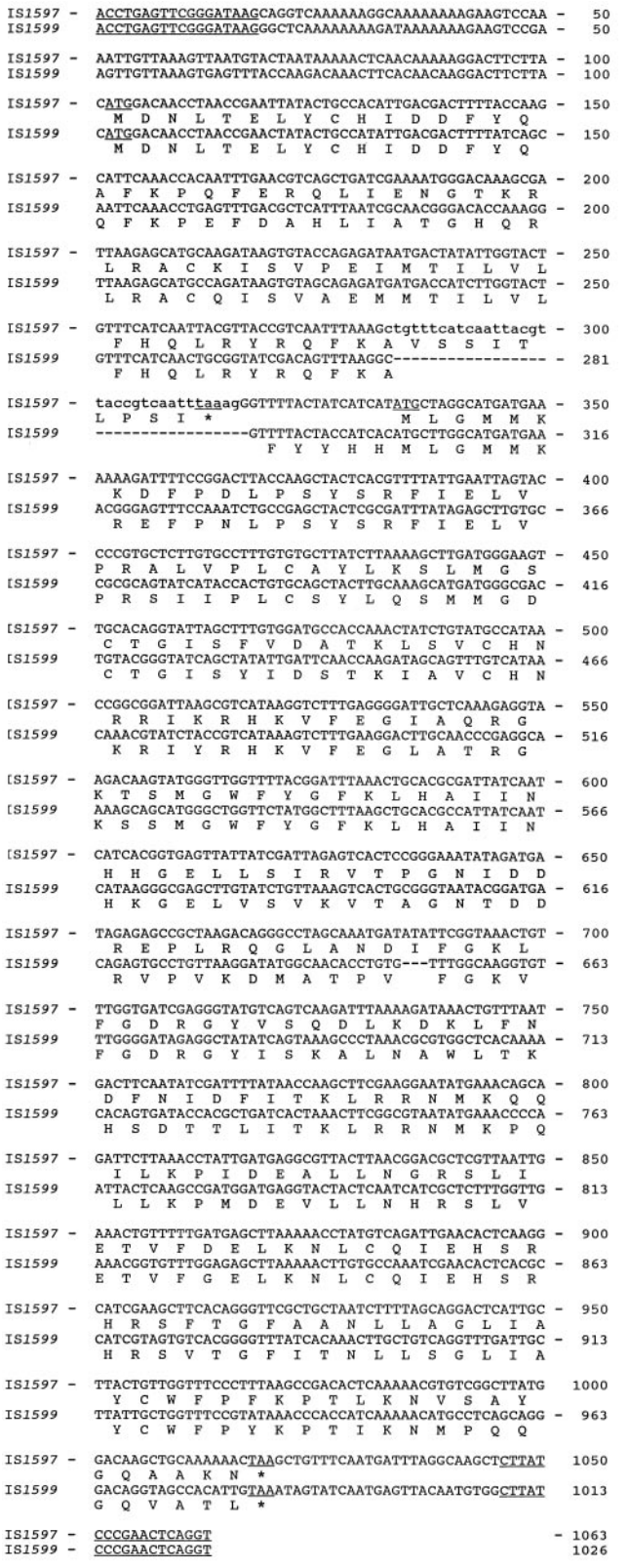

FIG. 1. DNA and amino acid sequence comparison between IS*1597* and IS*1599*, the IS sequence described in this study. The 18-bp inverted repeats at both ends of the IS sequences and translational start and stop codons are underlined. The 34-bp direct repeat in IS*1597* is displayed in lowercase letters. The dashes in the IS*1599* sequence indicate missing bases compared to the IS*1597* sequence.

(5, 23). The *tet*(34) and *tet*(35) genes have recently been described in *Vibrio* spp. (16, 23). In the present study, the *tet*(34) gene was found in *S. liquefaciens* and *P. pseudoalcaligenes* along with the *tet*(B) gene, and both isolates came from the same farm. Whether these two genes are on the same plasmid in each host and whether the plasmids from each species are related to each other is under investigation. Initial Southern blots show both the *tet*(34) and *tet*(35) genes hybridizing with the chromosomal DNA in the original isolates and their transconjugants. Clearly more work needs to be done with these isolates. The *S. maltophilia* isolate carrying the *tet*(35) gene was also isolated in this fish farm. These data extend the host range, since both isolates came from the same farm, and suggest that both *tet*(34) and *tet*(35) genes may be common in bacteria of cold water animals and their environment.

The *tet*(H) gene has previously been identified as part of the small composite transposon Tn*5706* (12), which was found in a complete or truncated form either on plasmids or in the chromosomal DNA of *Pasteurella* and *Mannheimia* spp. The finding that the *tet*(H) gene was present in isolates of *Acinetobacter radioresistens* and *Moraxella* sp. from salmon farms was the first detection of this gene in bacteria other than *Pasteurella* and *Mannheimia*. Sequence analysis of a 1,076-bp fragment, which comprised almost the entire *tet*(H) gene, revealed no differences in the nucleotide sequence of the *tet*(H) gene (10–12). Copies of the Tn*5706*-associated insertion sequence IS*1597* were present on the *tet*(H)-carrying plasmid in *A. radioresistens*. A novel IS element, IS*1599*, lacked a 34-bp direct repeat present found in IS*1596*/*1597* and exhibited only a single open reading frame for a putative transposase protein of 298 aa. The IS*1599*-borne transposase protein showed highest similarity to the 228-aa transposase proteins of IS*1596*/*1597* and was considered to be a member of the same family of IS elements.

Tc^r bacteria from fish bacteria in previous studies have had a variable ability to transfer the Tc^r phenotype, with the exception of the *tet*(E) gene, which has been associated with nonconjugative plasmids (5, 23). The strains carrying the *tet*(A), *tet*(H), and *tet*(L) genes, as well as those carrying one of the unknown genes, did not transfer, while the strain carrying both *tet*(B)and *tet*(34) transferred both genes, and the strain carrying *tet*(35) transferred the Tc^r phenotype, but not the *tet*(35) gene, suggesting that a novel *tet* gene may also be present in this isolate. Unlike the two isolates carrying *tet*(H) in this study, previously described *tet*(H)-carrying strains carry plasmids that replicate and express the Tc^r phenotype in *Pasteurella*, *Mannheimia*, and *E. coli* recipients (10–12). The *tet*(H) gene is part of a transposon (12) that might have integrated into a limited-host-range plasmid of *A. radioresistens* and *Moraxella*, which are replication deficient in *Pasteurella*, *Mannheimia*, and *E. coli* recipients, which could account for the lack of Tc^r transformants in the transformation and electrotransformation experiments conducted in this study.

In summary, the data from this study showed that gram-negative bacteria from the salmon farm environment harbor a variety of *tet* genes. A number of new genera were found to carry known *tet* genes, while 10 isolates may carry novel tetracycline resistance genes. The finding of the *tet*(H), *tet*(L), *tet*(34), and *tet*(35) genes in gram-negative bacteria from these farms extends our knowledge on the distribution of *tet* genes and suggests that a wide spectrum of *tet* genes, rather than the genes *tet*(A) to *tet*(G), should be used when future studies are done. Clearly surveillance studies of fish farms and other food-producing farms outside of Japan, Europe, and North America are needed to monitor the continuing evolution in the distribution of *tet* genes in this environment.

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